AGRICULTURAL NOTES

PUBLISHED BY
PORTO RICO AGRICULTURAL EXPERIMENT STATION, MAYAGUEZ
OFFICE OF FARM MANAGEMENT, FEDERAL BUILDING, SAN JUAN

No.32 Page 1. San Juan, Porto Rico, October, 1926.

SOME PINEAPPLE PROBLEMS.

16th ARTICLE. - ENZYMES IN THE LEAVES.

By Henry C. Henricksen.

In many chemical processes the reaction is slow until some substance is added which serves to accelerate it. Such substances, when inorganic, are called catalysts; they serve as accelerators only, that is they do not enter into the products formed. When the accelerators are of an organic nature, they are called enzymes and several such are present in plants.

The role of enzymes in the plant's economy is not always readily definable, but the presumption is that the reaction in the plant is similar to what it is outside. When that presumption is correct, enzyme determination may furnish information that is helpful in explaining some of the differences encountered in the plant.

The enzymes considered in this investigation are oxidase, peroxidase, catalase, reductase and diastase. Oxidase was found to be practically absent from all parts of the plant. A small amount was found in the tissue of the cut surface of green fruit after exposure to the air for several days. But aside from that the amount present was found to be too small for quantitative determination.

Peroxidase. - This is present in abundance in all parts of the plant. The quantity found in the leaves by Gile (Bulletin No.11, this Station) was verified, showing that green leaves generally contain more than chlorotic leaves. But as a whole the measurements of peroxidase did not show very striking differences in plants that were different, one from another, in color and in other respects.

Catalase. - Measurements of this were found to furnish valuable information and for that reason the procedure will be described. One half gram or three disks, cut from the fresh leaf with a 10 mm cork borer, was used. The latter is the more reliable when comparing one plant with another, for a given leaf area from different plants differs in weight, chiefly according to the thickness of the water-storing layer and that contains no catalase. The tissue was shredded fine, with a sharp knife into a small agate mortar and about 1/2 gram precipitated chalk was added together with enough water to make a thin paste. The mass was then well pestled after which it was washed into a large test tube with water and the volume made to 10 cc. A few drops toluol was added and the tube was left exposed to the air for about 18 hours.

The determination was made in a three-pronged tube, similar to that described by Heinicke, Memoir No.74, Cornell Agricultural Experiment Station, in the following manner: The 10 cc. tissue-chalk mixture was poured into one prong of the tube and

10 cc. of a 3% hydrogen peroxide into the other after which the third prong was connected with a burette and the water level adjusted. The temperature of the tube was brought to 26°C in a waterbath after which it was turned in the waterbath, so as to mix the contents of the two prongs every two seconds. The oxygen liberated from the peroxide was measured in the burette and the time required for the liberation of different volumes was noted. The 3% peroxide is not the most desirable but, as it is the grade handled by dealers in Porto Rico, it is the most practical, for fresh stock can be obtained. Whichever grade is used it must be not more than a few weeks old and the content of the bottle must be used a few hours after it is uncorked.

TYPICAL RESULTS WITH LEAVES.

No.	Material.	Minutes required for the liberation of 5 cc.	10 cc.	20 cc.
27	Plant from plat 43 (no phosphate) Very vigorous judged by color, pH and nitrate tests			
	Mature leaf	.75	2.25	7.
	Duplicate sample	1.	2.5	7.
	Duplicate sample	1.	2.	6.
27A	Another leaf, former plant	.75	1.75	5.5
	Duplicate sample	.75	1.5	4.5
	Duplicate sample	.75	1.5	4.5
27B	S t a l k	4.	10.	
59	Fruiting plant, appearing to be one hundred per cent normal	.75	1.5	6.
	Another leaf same plant	1.	2.	7.
60	Plant from same field as No.59 Leaves slightly more reddish	1.5	2.25	10.
	Another leaf same plant	1.	2.25	9.
	Another leaf same plant	1.	2.25	10.5
61	Plant from same field as former much smaller and leaves light in color		.8 cc.	
		3.5	12.	
	Another leaf same plant	4.25	14.	
	Another leaf same plant	4.5	18.	

The reliability of the method is illustrated in Nos. 27 and 27A. Duplicate samples from any one leaf give practically duplicate results, but there may be some variation if the samples are not taken from about the same position of the leaf. About the center is the best place, for the content increases slightly from base to tip. Different leaves from the same plant may vary, as shown in the table, although the variation is frequently much greater than the figures there given, which seems to be the case especially when the condition of the plant is in a state of change. The catalase content of the stalk is shown in No. 27B. It is typical of normal plants, the content being lower in the leaf bases than in the green leaves and still lower in the stalk.

The correlation of catalase content of the leaves with the general appearance of the plant is illustrated in Nos. 59, 60 and 61. The first-mentioned is a plant

which most growers would judge to be nearly perfect. The next one appeared to be much less perfect yet the catalase content is not much depressed. The third one is a typical poor plant, of which there are always some in most fields. The cause may be root injury, unsuitable soil conditions or perhaps a potentially poor plant. The catalase content shows that it is functioning much slower than those that appear to be normal.

While the examples given are typical, the figures from catalase determinations obtained with plants from one field cannot be compared with those from another field, without some knowledge of previous treatment. Also it is necessary to be well familiar with the various differences in plants and the factors causing them. The difference between temporary dormancy and permanent senescence is not readily detected with a catalase determination. Neither is it a safe guide to fertilizing without knowing when the field was fertilized previously and whether or not the weather has been such as to allow the plants to absorb it. But one who is well familiar with the various abnormal conditions, found from time to time, will find catalase determinations to be a valuable aid in solving his problems.

Reductase. - What is here described as reductase may not be an enzyme for it is not destroyed by boiling, but whatever it is it reduces nitrates to nitrites outside the plant and presumably also in the plant. In this investigation it was determined in the following manner: The fresh material was ground in a food chopper and weighed. The mass was squeezed in a muslin bag and washed with water three consecutive times after which water was added to make the volume of the juice 2 cc. for each gram of ground-up material. It was then boiled and filtered. (The boiling facilitates filtering without impairing the reducing power of the juice). After cooling, the acidity of the juice was determined on a small sample after which the reaction of the main portion was adjusted to pH 7.6 by the addition of a potassium hydroxide solution.

As reagent, a 4% potassium nitrate was used. This was first boiled with a small amount of hydrogen peroxide to oxidize all nitrite present. Two cc. of that was taken in a test tube together with 10 cc. of the plant juice. The tube was inserted in a water-bath at 50°C for 5 minutes after which the nitrite color was developed with a sulphanilic acid-naphylamine solution and determined in a Duboscq colorimeter. The results showed that the leaf of a very chlorotic plant contained scarcely a trace of reductase whereas one of a normal green plant contained an abundance. Between the two extremes the amount found generally paralleled that of the chlorophyll content. Aside from that the age of the leaf was also found to affect the content, the young leaf containing less than the fully mature leaf.

The results are interesting when compared with the nitrate content of the leaf. It was stated in Article No. 15 that the chlorotic leaf contains an abundance of nitrates almost to the tip, whereas a normal green leaf is devoid of nitrates a few inches above the white leaf-base, unless it is growing very vigorously. This

difference may be explained, perhaps, as being due to the reductase content. That is, perhaps the nitrates persist in the chlorctic plant because reductase is absent On the other hand it was stated that an abundance of nitrate is found in the white leaf-bases but that the content diminishes inch by inch in the green part, indicating that something is present in the latter which is absent in the former. That, however, could not be reductase for the tests show that the stalk and leaf-base contain six to eight times more than the green leaf. That would indicate the presence of some factor interferring with reduction in the white tissue. It may be due, in part, to the acidity. Reductase does not reduce well in an acid solution and the leaf base and stalk is practically always more acid than the leaf.

Diastase. - This enzyme, or perhaps a mixture of several enzymes, converts raw starch into a soluble form, that again into dextrine and the dextrine into sugar. In preliminary trials it was found to be present in all parts of the pineapple plant. It was determined quantitatively as follows: the material was prepared as described under reductase but not boiled, for boiling destroys this enzyme. Twenty cc. of the filtered juice, corresponding to 10 grams fresh tissue, was taken, in a large test tube, and 25 milligrams boiled starch, in the form of a thin paste, was added. The tube was then inserted in a water-bath at 40°C and 2 cc. samples were withdrawn, from time to time, and tested for starch with iodine dissolved in potassium iodide. The results showed that under the conditions described the leaves of plants six to twelve months old generally contained enough diastase to convert the starch into dextrine in about 20 minutes and the dextrine into sugar in about 10 minutes more. Plants were found to vary very much, one from another, in respect to diastase content. Fruiting plants or plants from which fruit had recently been picked usually contained twice as much as the amount noted above. Young, very vigorous plants, on the other hand, contained but one-fourth to one-half that amount. This would indicate that diastase is formed in greater quantity in older leaves and especially in leaves at the time the plant is blooming and fruiting. Also that diastase is perhaps a contributing factor to fruit formation. Yet the stalk of a fruiting plant, or one showing signs of blooming contains no more diastase than that of a non-blooming plant.

The most conspicuous facts are that the leaves of pineapple plants contain an abundance of diastase, enough to convert the starch, formed from photosynthetic cugar, at any time. The stalk contains much less than the leaves but as there is no consistent variation between stalks from different plants there is nothing to indicate that this condition is abnormal.